

WHAT IS CLAIMED IS:

- 1 1. A method for simultaneous detection and/or determination of a plurality of
2 modified proteins in a sample, comprising:
 - 3 a) contacting the sample under mild protein denaturation conditions with a plurality of
4 first antibodies capable of binding to a specific target protein, the first antibodies
5 being immobilized on solid support material, each first antibody being differentiable
6 from others by a differentiation parameter, whereby the first antibodies bind to
7 respective target proteins present in the sample;
 - 8 b) removing unbound materials from the locus of the first antibodies;
 - 9 c) contacting the materials from step (b) with one or more second antibodies, each of
10 which is specific to a class or subclass of modified proteins or with a plurality of
11 second antibodies, each of which is specific to a modified protein, so as to bind the
12 second antibody or antibodies to modified proteins in the sample; and
13 d) detecting and/or determining a plurality of modified proteins in the sample.
- 1 2. A method according to claim 1, wherein up to 100 modified proteins are
2 detected and/or determined.
- 1 3. A method according to claim 1 wherein the modified proteins are selected
2 from phosphorylated proteins, glycosylated proteins, acetylated proteins, methylated proteins,
3 ubiquinated proteins, and prenylated proteins.
- 1 4. A method according to claim 3 wherein the modified proteins are
2 phosphorylated proteins.
- 1 5. A method according to claim 1 wherein the solid support material comprises a
2 series of subsets of solid particles, each subset being distinguishable from other subsets in
3 accordance with a particular property or characteristic.
- 1 6. A method according to claim 5 in which the solid particles are differentiable
2 by specific color or emission spectra.

1 7. A method according to claim 5 in which the solid particles comprise spherical
2 particles formed from non-porous glass, polystyrene or latex.

1 8. A method according to claim 1 in which the solid support material is a
2 microchip, a plate having a multiplicity of wells, or a slide.

1 9. A method according to claim 1 wherein the materials from step (b) are
2 contacted in step (c) with one or more second antibodies, each of which is specific to a class
3 of modified proteins.

1 10. A method according to claim 9 in which the materials from step (b) are
2 contacted in step (c) with a second antibody that is specific to a class of modified proteins.

1 11. A method according to claim 1 in which the materials from step (b) are
2 contacted in step (c) with a second antibody that is specific to a subclass of modified proteins.

1 12. A method according to claim 1 in which the materials from step (b) are
2 contacted in step (c) with one or more second antibodies specific to phosphorylated proteins.

1 13. A method according to claim 1 in which the materials from step (b) are
2 contacted in step (c) with a plurality of second antibodies, each of which is specific to a
3 modified protein.

1 14. A method according to claim 1 in which the materials from step (b) are
2 contacted in step (c) with a plurality of second antibodies, each of which is specific to a
3 phosphorylated protein.

1 15. A method according to claim 14 in which the proteins are selected from
2 phosphorylated p38MAPK, phosphorylated I κ B α , phosphorylated Erk2, phosphorylated JNK
3 and phosphorylated Akt.

1 16. A method according to claim 1 in which the second antibodies are biotinylated
2 antibodies.

1 17. A method according to claim 1 in which the modified proteins are detected
2 and/or determined in step (d) by contacting the product of step (c) with a labeled moiety.

1 18. A method according to claim 17 in which the labeled moiety comprises a
2 phycobiliprotein.

1 19. A method according to claim 17 in which the labeled moiety comprises a
2 phycoerythrin.

1 20. A method according to claim 17 in which the labeled moiety comprises a
2 conjugate of a labeled moiety with streptavidin.

1 21. A method according to claim 1 in which the sample is a cell lysate.

1 22. A method according to claim 1 in which the sample is contacted with a sulfate
2 or sulfonate detergent in step (a).

1 23. A method according to claim 22 in which the detergent is sodium dodecyl
2 sulfate.

1 24. A kit for simultaneous detection and/or determination of a plurality of
2 modified proteins in a sample, comprising:

3 (a) a plurality of first antibodies, each capable of binding to a specific target
4 protein, each first antibody being immobilized on a solid support material and each
5 first antibody being differentiable from others by a differentiation parameter;

6 (b) one or more buffers for lysing and for washing cellular material samples to be
7 assayed

8 (c) an assay buffer for conducting the assay, said buffer containing from about 1-10
9 mM of a sulfate or sulfonate detergent; and

10 (d) one or more second antibodies specific to classes or subclasses of modified
11 proteins or to specific individual modified proteins.

1 25. A kit according to claim 24 wherein the solid support material comprises a
2 series of subsets of solid particles, each subset being distinguishable from other subsets in
3 accordance with a particular property or characteristic.

- 1 26. A kit according to claim 25 in which the solid particles are differentiable by
2 specific color or emission spectra.
- 1 27. A kit according to claim 25 in which the solid particles comprise spherical
2 particles formed from non-porous glass, polystyrene or latex.
- 1 28. A kit according to claim 24 in which the solid support material is a microchip,
2 a plate having a multiplicity of wells, or a slide.
- 1 29. A kit according to claim 24 wherein the modified proteins are selected from
2 phosphorylated proteins, glycosylated proteins, acetylated proteins, methylated proteins,
3 ubiquinated proteins, and prenylated proteins.
- 1 30. A kit according to claim 24 wherein the modified proteins are phosphorylated
2 proteins.
- 1 31. A kit according to claim 24 wherein the second antibodies comprise one or
2 more antibodies that are specific to classes of modified proteins.
- 1 32. A kit according to claim 24 wherein the second antibodies comprise one or
2 more antibodies that are specific to subclasses of modified proteins.
- 1 33. A kit according to claim 24 wherein the second antibodies are specific to
2 phosphorylated proteins.
- 1 34. A kit according to claim 24 wherein the second antibodies comprise a plurality
2 of antibodies, each of which is specific to an individual modified protein.
- 1 35. A kit according to claim 24 further comprising a labeled moiety.
- 1 36. In a process for simultaneously analyzing a sample for a plurality of modified
2 proteins, the step of denaturing modified proteins comprising contacting the sample with a
3 sulfate or sulfonate detergent, preferably in a concentration of about 1-10 mM, at a
4 temperature of between about 4 and about 37 °C, and for a time of from about 2 to about 72
5 hours.
- 1 37. A process according to claim 36 in which the detergent is sodium dodecyl
2 sulfate.